REMARKS

Claims 1-8, 10-14 and 16-35 are pending. Claims 1-6, 8, 10-14 and 16-17 have been amended. Claim 15 has been cancelled. New claims 18-35 have been added. Support for the claim amendments and new claims can be found throughout the present application. No new matter has been added.

Rejection of Claims 16 and 17 under 35 U.S.C. §101

Claims 16 and 17 are rejected under 35 U.S.C. §101 "because the claimed invention is directed to non-statutory subject matter."

The claims have been amended to reflect that the transgenic mammal is a non-human mammal, thereby obviating this rejection.

Rejection of Claims 14-16 Under 35 U.S.C. §112, second paragraph

Claims 14, 15 and 16 are rejected under 35 U.S.C. §112, second paragraph "as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention."

Claim 15 has been cancelled and claims 14 and 16 have been amended to remove the informalities in these claims, thereby obviating this rejection.

Rejection of Claims 1-8 and 10-17 Under 35 U.S.C. §103

Claims 1-8 and 10-17 are rejected under 35 U.S.C. §103 as being "unpatentable over U.S. Patent No. 5,959,171 ... hereafter referred to as Hyttinen et al., in view of Zewe et al. (1997) Immunotech., Vol. 3(2), 127-136. According to the Examiner,

> Hyttinen et al. teaches vectors encoding a fusion protein operatively linked to regulatory elements needed for high level mammary gland specific expression derived from a milk protein gene or a mammary tumor virus and a DNA sequence encoding a signal sequence needed for secretion and maturation of the fusion protein ... Hyttinen also teach transgenic animals made using said vectors, and methods of making a fusion protein comprising collecting milk from a transgenic mammal which expresses a fusion protein in its milk, and isolating the

recombinant fusion protein from the milk ... Hyttinen et al. further teaches that making and using a transgenic mouse which expresses a beta-lactoglobulin-hEPO fusion protein at concentrations of 0.2-1 mg/ml in the transgenic milk ... Hyttinen et al. also teaches that the general idea of making and using transgenic bioreactors for the production of large quantities of proteins, particularly human proteins, was suggested in the art as early as 1986 and that numerous examples of transgenic bioreactors exist in the art ... Thus, Hyttinen establishes that the art recognized the advantages of producing large quantities of biologically relevant, therapeutic proteins in the milk of transgenic animals.

Although Hyttinen et al. teaches general methods for making transgenic animals comprising fusion proteins and methods of making and isolating fusion proteins from the milk of transgenic mammals, Hyttinen et al. differs from the instant invention by not specifically teaching the production of a fusion protein comprising angiogenin. Zewe et al. supplements Hyttinen et al. by teaching nucleic acid expression constructs which encode a fusion protein comprising a single chain antibody against the transferrin receptor and angiogenin. ... While Zewe et al. teaches the expression of the fusion protein in bacteria, the skilled artisan would have been motivated to express the fusion protein taught by Zewe et al. using a mammalian bioreactor system in order to produce larger quantities of human fusion protein as taught by Hyttinen et al., and in order to avoid the contamination of the fusion proteins with bacterial toxins. ... Therefore, in view of the benefits of using a transgenic bioreactor to produce large quantities of a protein for use in humans, it would have been prima facie obvious to a skilled artisan to express the fusion protein taught by Zewe et al. using the transgenic bioreactor taught by Hyttinen. Further, based on successful use of transgenic bioreactors in expressing large quantities of a variety of human proteins and fusion proteins as taught by Hyttinen et al., the skilled artisan would have had a reasonable expectation of success in expressing a fusion protein comprising a single chain antibody against the transferring receptor and angiogenin in the milk of a transgenic mammal according to the methods taught by Hyttinen et al.

Applicants respectfully traverse this rejection. The claims, as amended, are directed to methods of making a fusion protein in the milk of a transgenic mammal, and to transgenic mammals producing such fusion proteins. The fusion protein includes an enzyme portion which is produced in the milk of the transgenic mammal in active form, and the fusion protein is produced at high levels, e.g., at least about 0.1 mg/ml, in the milk.

Hyttinen et al. disclose producing a fusion protein in inactive form in the milk of a transgenic animal. Specifically, Hyttinen et al. disclose that there can be "severe side effects" when "producing potent polypeptides like growth factors, cytokines or enzymes" in the milk of

transgenic mammals. To solve such problems, Hyttinen et al. disclose producing such polypeptides as part of a fusion protein such that the polypeptide is produced in the milk of the animal in inactive form. (emphasis added). Thus, it is clear that nothing in the Hyttinen et al. reference teaches or suggests producing a fusion protein which includes an enzyme such that the enzyme is produced in the milk of a transgenic animal in active form. In addition, nothing in the teachings of Hyttinen et al. would motivate a skilled artisan to produce fusion proteins in which the enzyme portion is in active form. This is contrary to what is suggested by Hyttinen et al.

Zewe et al. disclose producing fusion proteins, which include various RNases and a single chain antibody to the transferrin receptor, in bacterial cells. Zewe et al. disclose that such fusion proteins are not secreted from the cells (see, e.g., pages 129-130, paragraph 2.3), and even after recovering such fusion proteins from the cells, the fusion proteins are produced at very low levels, i.e., about .47 μg/ml to about 3.1 μg/ml, (see page 131-132, paragraph 3.2). Thus, Zewe et al. do not teach or suggest producing such fusion proteins in the milk of a transgenic mammal.

In addition, the fact that the fusion proteins disclosed by Zewe et al. are not secreted would hardly motivate a skilled artisan to produce such proteins in a system in which secretion is an essential step in recovering the fusion protein.

Moreover, in view of the very low expression levels of these fusion proteins in cell culture (see, e.g., Zewe et al.), it was unexpected that these proteins would be secreted at such high levels in the milk of transgenic mammals.

Thus, it is clear that neither Hyttinen et al. nor Zewe et al, alone or in combination, teach or suggest the claimed invention. Moreover, there is nothing in either of these references which would motivate a skilled artisan to combine the teachings of these reference to arrive at the claimed invention. Lastly, the expression levels of the fusion protein obtained in the milk of transgenic mammals, and presently claimed, were unexpected in view of the very low expression levels of these proteins in other expression systems. Therefore, the Hyttinen et al. and Zewe et al. references do not render the claimed invention obvious, and Applicants respectfully request that the Examiner withdraw this rejection.

Attached is a marked-up version of the changes being made by the current amendment.

Applicant asks that all claims be allowed. Enclosed is a check for excess claim fees and a check for the Petition for Extension of Time fee. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 13 Jan 02

Reg No. 35965

Laurie Butler Lawrence
Reg. No. 46,593

Fish & Richardson P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

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Version with markings to show changes made

In the claims:

Claim 15has been cancelled.

Claims 1-6, 8, 10-14 and 16-17 have been amended as follows:

1. (Amended) A method of making a transgenic fusion protein which includes a first member and a second member, wherein the second member is an enzyme, the method comprising: providing a non-human transgenic [animal] mammal which includes a transgene which provides for the expression of the fusion protein in the milk of the mammal; and allowing the transgene to be expressed[; and recovering the fusion protein for the milk of the transgenic animal], thereby providing the fusion protein in the milk of the mammal wherein the second member is in active form and the fusion protein is produced at levels of at least about 0.1 mg/ml in the milk of the mammal.

2. (Amended) The method of claim 1, wherein the [fusion protein includes] first member of the fusion protein is an immunoglobulin subunit [and an enzyme].

3. (Amended) The method of claim 1, wherein the [fusion protein includes a] first member is fused to [a] the second member and the first member includes [the] a subunit of a targeting molecule and the second member encodes a cell toxin.

4. (Amended) The method of claim 1, wherein the [fusion protein] first member includes a subunit of an [Ig] immunoglobulin specific for a tumor antigen.

5. (Amended) The method of claim 4, wherein the tumor antigen is from the group consisting of carcinoembryonic antigen (CEA), a [transferring] transferrin receptor, TAG-72, and an epidermal growth factor.

6. (Amended) The method of claim 1, wherein the [fusion protein includes] second member is an RNase.

8. (Amended) The method of claim 1, wherein the [fusion protein includes] second member is angiogenin.

10. (Amended) The method of claim [1] 2, wherein the [fusion protein is made in a mammary gland of the transgenic mammal] immunoglobulin subunit of the fusion protein is a human antibody or antigen binding portion thereof.

11. (Amended) The method of claim 1, wherein the fusion protein is [secreted into] produced in the milk of [a transgenic] the mammal at concentrations of at least about 0.5 mg/ml [or higher].

12. (Amended) The method of claim 1, wherein the fusion protein is [secreted into] produced in the milk of a transgenic mammal at concentrations of at least about 1.0 mg/ml.

13. (Amended) The method of claim [1] 2, wherein the immunoglobulin subunit of [a] the fusion protein is a humanized antibody or antigen binding portion thereof.

14. (Amended) The method of claim 1, wherein the transgene encoding the [transgenic] fusion protein is a nucleic acid [construct] which [includes] comprises:

(a) [optionally, an insulator sequence;

(b)] a mammary epithelial specific promoter;

[(c)] (b) a nucleotide sequence which encodes a signal sequence which can direct the secretion of the fusion protein [e.g., a signal from a milk protein;

(d) optionally, a nucleotide sequence which encodes a sufficient portion of the amino terminal coding region of a secreted protein, e.g., a protein secreted into milk, to allow secretion, e.g., in the milk of a transgenic mammal, of the fusion protein; and

(e)] (c) one or more nucleotide sequences which encode the fusion protein[; and

(f) optionally, a 3' untranslated region from a mammalian gene].

16. (Amended) A non-human transgenic [animal] mammal which includes a transgene that encodes a fusion protein [described in claim], the transgene comprising: a mammary epithelial specific promoter, a nucleotide sequence which encodes a signal sequence which can direct the secretion of the fusion protein, and one or more nucleotide sequences encoding the fusion protein, wherein the fusion protein includes a first member and a second member, the second member is an enzyme produced in the milk of a transgenic mammal in active form, and the fusion protein is produced in the milk of the transgenic mammal at a concentration of at least about 0.1 mg/ml.

17. (Amended) The transgenic [animal] mammal of claim [15] 16, which can [secrete] produce the fusion protein into its milk at concentrations of at least about [0.5 mg/mll or higher] 0.5 mg/ml.